

patched loss-of-function phenotype and identifying those test agents that affect the patched loss-of-function phenotype.

63. **(Twice Amended)** A method for identifying an agent which affects patched-dependent signal transduction in a cell characterized by loss of function of a patched gene, comprising:

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- a. comparing the amount of expression of a reporter gene in a first recombinant mammalian cell in the presence of a test agent with the amount of expression in the absence of the agent, or with the amount of reporter gene expression in a second recombinant cell; and
- b. identifying test compounds that decrease the amount of expression of the reporter gene in the first recombinant cell in the presence of the agent compared to the amount of expression in the absence of the agent, or compared to the amount of reporter gene transcription or product in the second recombinant cell, wherein:

the first recombinant cell contains a reporter gene construct and expresses patched;

the second recombinant cell is identical to the first recombinant cell, except that it does not express a functional wild-type patched protein; and

the reporter gene construct contains:

- (i) a transcriptional control element that is stimulated by a patched-dependent intracellular signal that is generated by the interaction of a hedgehog protein with patched; and
- (ii) a reporter gene that encodes a detectable product and that is in operative association with the transcriptional control element;

wherein a test agent identified in step (b) affects patched-dependent signal transduction in a cell characterized by loss of function of a patched gene.

64. **(Reiterated)** The method of claim 63, wherein the amount of transcription is determined by measuring the amount of mRNA that is transcribed from said reporter gene.

65. **(Reiterated)** The method of claim 63, wherein the amount of transcription is measured by measuring the amount of reporter gene protein that is produced.

66. **(Reiterated)** The method of claim 63, further comprising, prior to comparing the difference in the amount of transcription of the reporter gene, contacting the recombinant cell

N.I.B.
with a hedgehog agonist in an amount sufficient to change the level of transcription of said reporter gene.

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67. (Amended) The method of claim 63, wherein the reporter gene is at least one of a gene encoding chloramphenicol acetyltransferase, a gene encoding firefly luciferase, a gene encoding bacterial luciferase, or a gene encoding alkaline phosphatase.

D
68. (Amended) The method of claim 63, wherein the transcriptional control region includes at least one regulatory element selected from transcriptional regulatory elements of a patched gene, transcriptional regulatory elements of a gli gene, or transcriptional regulatory elements of a PTHrP gene.

69. (Reiterated) The method of claim 63, wherein the patched protein is encoded by a nucleic acid which hybridizes at 5 x SSC at 65° C to SEQ ID No. 18.

70. (Reiterated) The method of claim 63, wherein expression of the reporter gene occurs upon hedgehog stimulation, and compounds are selected by ability to inhibit the patched-dependent expression of the reporter gene.

N.P.
71. (Reiterated) The method of claim 63, wherein the cell having a patched loss-of-function phenotype is a basal cell carcinoma.

72. (Reiterated) A method for preparing an agent for inhibiting growth of cells characterized by loss of function of a patched gene, comprising:

- a. contacting one or more test agents with a cell that expresses a wild-type patched protein and identifying test agents that change the level of patched-dependent intracellular signal transduction relative to the absence of test agent;
- b. contacting test agents identified in step (a) with a cell having a patched loss-of-function phenotype and selecting those test agents that reverse at least in part the patched loss-of-function phenotype; and
- c. preparing a formulation including a test agent that inhibits the growth of cells selected in step (b) and a pharmaceutically acceptable diluent.

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73. (Reiterated) The method of claim 72, wherein the cell having a patched loss-of-function phenotype is a basal cell carcinoma cell.

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74. (Amended) The method of claim 61 or 62, further comprising preparing a formulation including an agent which affects patched-dependent signal transduction and a pharmaceutically acceptable excipient.

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75. (Reiterated) The method of claim 74, further comprising administering the formulation to a patient.

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76. (Amended) The method of claim 63, further comprising preparing a formulation including an agent which affects patched-dependent signal transduction and a pharmaceutically acceptable excipient.

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77. (Reiterated) The method of claim 76, further comprising administering the formulation to a patient.

The amended claims are re-stated below to reflect changes from the last filing.

61. (Twice Amended) A method for identifying an agent which affects patched-dependent signal transduction [for ameliorating an effect of loss of function of a patched gene] in a cell, comprising contacting one or more test agents with a cell that expresses a wild-type patched protein and identifying test agents that [change]affect the level of patched-dependent[inhibited intracellular] signal transduction relative to the absence of test agent[, wherein agents that cause a decrease in patched inhibited signal transduction are useful for ameliorating an effect in a cell characterized by loss of function of a patched gene].

62. (Twice Amended) A method for identifying an agent which affects patched-dependent signal transduction [for ameliorating an effect of loss of function of a patched gene] in a cell, comprising contacting one or more test agents with a cell having a patched loss-of-function phenotype and identifying those test agents that [ameliorate at least in part] affect the patched

loss-of-function phenotype], wherein test agents that affect the patched loss-of-function phenotype are useful for ameliorating an effect in a cell characterized by loss of function of a patched gene].

63. (Twice Amended) A method for identifying an agent [for modulating proliferation] which affects patched-dependent signal transduction in a cell characterized by loss of function of a patched gene, comprising:

- a. comparing the amount of expression of a reporter gene in a first recombinant mammalian cell in the presence of a test agent [compound] with the amount of expression in the absence of the agent [compound], or with the amount of reporter gene expression in a second recombinant cell; and
- b. identifying test compounds that decrease the amount of expression of the reporter gene in the first recombinant cell in the presence of the agent [compound] compared to the amount of expression in the absence of the agent [compound], or compared to the amount of reporter gene transcription or product in the second recombinant cell, wherein:

the first recombinant cell contains a reporter gene construct and expresses patched;

the second recombinant cell is identical to the first recombinant cell, except that it does not express a functional wild-type patched protein; and

the reporter gene construct contains:

- (i) a transcriptional control element that is stimulated by a patched-dependent intracellular signal that is generated by the interaction of a hedgehog protein with patched; and
- (ii) a reporter gene that encodes a detectable product and that is in operative association with the transcriptional control element;

wherein a test agent [compound] identified in step (b) affects patched-dependent signal transduction [is useful for modulating proliferation] in a cell characterized by loss of function of a patched gene.

67. (Amended) The method of claim 63, wherein the reporter gene is [selected from the group consisting of] at least one of a gene encoding chloramphenicol acetyltransferase, a gene